```
CHO, BHK, 293 cells, Vero expressed interferon alpha-14C.

h = 1 to 3;
a-g, j-m, i (independently selected) = 0 or 1;
r-u (independently selected) = 0 or 1;
n, v-y = 0; z = 1.
```

1. CMP-SA-PEG, α2,8-ST

```
h = 1 to 3;
a-g, i, r-u (independently selected) = 0 or 1;
j-m (independently selected) = 0 to 2;
v-y (independently selected) = 1,
when j-m (independently selected) is 2;
z = 1; n = 0; R = PEG.
```

FIG. 30J

```
CHO, BHK, 293 cells, Vero expressed
Interferon alpha-14C.
a-g, j-m, r-u (independently selected) = 0 or 1;
h = 1 to 3; n, v-y = 0; z = 1.
```

- 1. Sialidase
- 2. Trans-sialidase, PEG-Sia-lactose

```
a-g, j-m, r-y (independently selected) = 0 or 1;
h = 1 to 3; n = 0; z = 1; R = PEG.
```

FIG. 30K

```
CHO, BHK, 293 cells, Vero expressed interferon alpha-14C.

h = 1 to 3;
a-g, j-m, i (independently selected) = 0 or 1;
r-u (independently selected) = 0 or 1;
n, v-y = 0; z = 1.

1. CMP-SA, α2,8-ST
```

```
h=1 to 3;
a-g, i, r-u (independently selected) = 0 or 1;
j-m (independently selected) = 0 to 40;
z=1; v-y, n=0.
```

FIG. 30L

```
Insect cell or fungi expressed interferon alpha-14C. a-d, f, h, j-n, s, u, v-y = 0; e, g, i, r, t (independently selected) = 0 or 1; z = 1.
```

- 1. GNT's 1 & 2, UDP-GlcNAc
- Galactosyltransferase, UDP-Gal-linker-SA-CMP
- 3. ST3Gal3, transferrin

a, c, e, g, i, r, t, v, x (independently selected) = 0 or 1; z = 1; b, d, f, h, j-n, s, u, w, y = 0; R = transferrin.

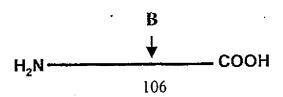
FIG. 30M

```
Insect cell or fungi expressed interferon alpha-14C.
a-d, f, h, j-n, s, u, v-y = 0;
e, g, i, r, t (independently selected) = 0 or 1; z = 1.
```

- 1. endoglycanase
- 2. Galactosyltransferase, UDP-Gal-linker-SA-CMP
- 3. ST3Gal3, transferrin

```
i (independently selected) = 0 or 1;
a-h, j-m, r-z = 0;
n = 1; R' = -Gal-linker-transferrin.
```

FIG. 30N



$$\mathbf{B} \leftarrow \begin{bmatrix} (\operatorname{GlcNAc-Gal})_{f_{c}}(\operatorname{Sia})_{b} - (R)_{g} \\ - \operatorname{GalNAc-(Gal})_{a} - (\operatorname{Sia})_{c} - (R)_{d} \end{bmatrix}_{e}$$

a-c, e, f (independently selected) = 0 or 1; d, g = 0; R = polymer, glycoconjugate.

FIG. 300

CHO, BHK, 293 cells, Vero expressed IF-alpha (2a or 2b).
a-c (independently selected) = 0 or 1;
e = 1; d, f, g = 0

- Sialidase
 - 2. CMP-SA-PEG, ST3Gal1

a-d (independently selected) = 0 or 1; e = 1; b, f, g = 0; R = PEG.

FIG. 30P

Insect cell expressed interferon alpha (2a or 2b). a, e (independently selected) = 0 or 1; b, c, d, f, g = 0.

- 1. Galactosyltransferase, UDP-Gal
 - 2. CMP-SA-PEG, ST3Gal1

a, c, d, e (independently selected) = 0 or 1; b, f, g = 0; R = PEG.

FIG. 30Q

E. coli expressed IF-alpha (2a or 2b). a-g = 0.

 GalNAc Transferase, UDP-GalNAc-PEG

a-c, f, g = 0; d, e = 1; R = PEG.

FIG. 30R

NSO expressed IF-alpha (2a or 2b). a (independently selected) = 0 or 1; e = 1; b, c, d, f, g = 0

1. CMP-SA-levulinate, ST3Gal1

2. H₄N₂-PEG

a, c, d (independently selected) = 0 or 1; e = 1; b, f, g = 0; R = PEG.

FIG. 30S

E. coli expressed IF-alpha (2a or 2b). a-g=0.

 Endo-N-acetylgalatosamidase (synthetic enzyme), PEG-Gal-GalNAc-F

a, d, e = 1; b, c, f, g = 0; R = PEG.

FIG. 30T

E. coli expressed IF-alpha (2a or 2b). a-g = 0.

- 1. GalNAc Transferase, UDP-GalNAc
- 2. sialyltransferase, CMP-SA-PEG

b, d = 0 or 1; e = 1; a, c, f, g = 0; R = PEG.

FIG. 30U

```
CHO, BHK, 293 cells, Vero expressed IF-alpha (2a or 2b).
a-c, f (independently selected) = 0 or 1;
e = 1; d, g = 0
```

- 1. Sialidase
- 2. CMP-SA-PEG, ST3Gal1 and ST3Gal3

```
a-d, f, g (independently selected) = 0 or 1;
e = 1; R = PEG.
```

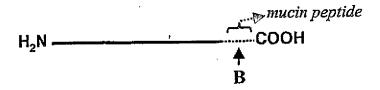
FIG. 30V

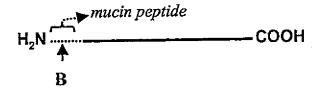
```
CHO, BHK, 293 cells, Vero expressed IF-alpha (2a or 2b). a-c, f (independently selected) = 0 or 1; e = 1; d, g = 0
```

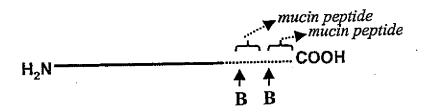
- 1. Sialidase
- CMP-SA-linker-SA-CMP, ,ST3Gal1
- 3. ST3Gal3, transferrin

a-d, f (independently selected) = 0 or 1; e = 1; R = transferrin; g = 0.

FIG. 30W







$$\mathbf{B} \leftarrow \begin{bmatrix} (\operatorname{Sia})_{b} \\ -\operatorname{GalNAc-(Gal)}_{a} - (\operatorname{Sia})_{c} - (R)_{d} \end{bmatrix}_{c}$$

a-c, e (independently selected) = 0 or 1; d = 0; R = polymer, glycoconjugate.

FIG. 30X

CHO, BHK, 293 cells, Vero expressed interferon alpha-mucin fusion protein. a-c, e (independently selected) = 0 or 1; d = 0

- 1. Sialidase
- 2. CMP-SA-PEG, ST3Gal1

a-d, e (independently selected) = 0 or 1; R = PEG.

FIG. 30Y

Insect cell expressed interferon alpha-mucin fusion protein.

a, e (independently selected) = 0 or 1; b, c, d = 0.

1. Galactosyltransferase, UDP-Gal-PEG

a, d, e (independently selected) = 0 or 1; b, c = 0; R = PEG.

FIG. 30Z

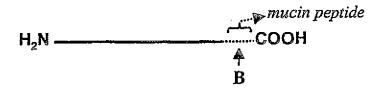
E. coli expressed interferon alpha-mucin fusion protein.

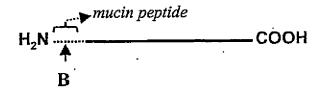
$$a-e = 0$$
.

- 1. GalNAc Transferase, UDP-GalNAc
- 2. CMP-SA-PEG, sialyltransferase

c, d, e (independently selected) = 0 or 1; a, b = 0; R = PEG.

FIG. 30AA





$$\mathbf{B} \quad \leftarrow \begin{bmatrix} (\operatorname{Sia})_{b} \\ & \\ -\operatorname{GalNAc-(Gal)}_{a} - (\operatorname{Sia})_{c} - (R)_{d} \end{bmatrix}_{e}$$

$$\mathbf{C} \leftarrow (\mathbf{R'})_{\mathbf{n}}$$

a-c, e (independently selected) = 0 or 1;d = 0; R = polymer, linker.

FIG. 30BB

E. coli expressed interferon alpha-mucin fusion protein.

a-e, n = 0.

 GalNAc Transferase, UDP-GalNAc-PEG

d, e (independently selected) = 0 or 1; a-c, n = 0; R = PEG.

FIG. 30CC

E. coli expressed interferon alpha-mucin fusion protein.

a-e, n = 0.

- GalNAc Transferase, UDP-GalNAc-linker-SA-CMP
- 2. ST3Gal3, asialo-transferrin
- 3. CMP-SA, ST3Gal3

d, e (independently selected) = 0 or 1; a-c, n = 0; R = linker-transferrin.

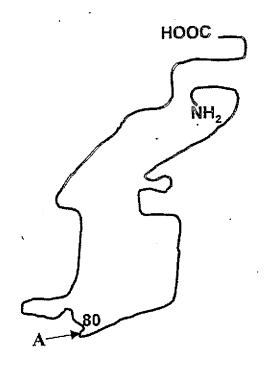
FIG. 30DD

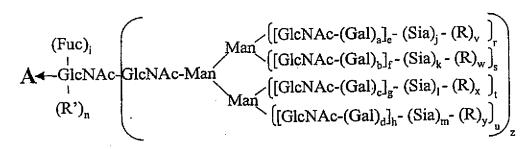
E. coli expressed Interferon alpha (no fusion). a-e, n = 0.

- 1. NHS-CO-linker-SA-CMP
- 2. ST3Gal3, transferrin

a-e=0; n=1; R'=linker-transferrin.

FIG. 30EE





a-d, i, r-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 4. j-m (independently selected) = 0 or 1. n, v-y = 0; z = 0 or 1; R = polymer

FIG. 31A

```
CHO, BHK, 293 cells, Vero expressed IF-beta h = 1 to 3; a-g, j-m, i (independently selected) = 0 or 1; r-u (independently selected) = 0 or 1; n, v-y = 0; z = 1.
```

1. Sialidase

2. CMP-SA-PEG, ST3Gal3

```
h = 1 to 3;
a-g, i (independently selected) = 0 or 1;
r-u (independently selected) = 0 or 1;
j-m, v-y (independently selected) = 0 or 1;
z = 1; n = 0; R = PEG.
```

FIG. 31B

```
Insect cell expressed IF-beta
a-d, f, h, j-n, s, u, v-y = 0;
e, g, i, r, t (independently selected) = 0 or 1;
z = 1.
```

- 1. GNT's 1&2, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal
- 2. CMP-SA-PEG, ST3Gal3, buffer, salt

```
b, d, f, h, k, m, n, s, u, w, y = 0;
a, c, e, g, i, r, t (independently selected) = 0 or 1;
j, l, v, x (independently selected) = 0 or 1;
z = 1; R = PEG.
```

FIG. 31C

```
Yeast expressed IF-beta
a-n = 0; z = 1;
r-y (independently selected) = 0 to 1;
R (branched or linear) = Man, oligomannose or polysaccharide.
```

- 1. Endo-H
- 2. Galactosyltransferase, UDP-Gal
- 3.. CMP-SA-PEG, ST3Gal3

a-m, r-z=0; n = 1; R' = -Gal-Sia-PEG.

FIG. 31D

```
CHO, BHK, 293 cells, Vero expressed IF-beta h = 1 to 3; a-g, j-m, i (independently selected) = 0 or 1; r-u (independently selected) = 0 or 1; n, v-y = 0; z = 1.
```

1. CMP-SA-PEG, ST3Gal3

```
h = 1 to 3;
a-g, i (independently selected) = 0 or 1;
r-u (independently selected) = 0 or 1;
j-m, v-y (independently selected) = 0 or 1;
z = 1; n = 0; R = PEG.
```

FIG. 31E

Insect cell expressed IF-beta a-d, f, h, j-n, s, u, v-y = 0; e, g, i, r, t (independently selected) = 0 or 1; z = 1.

- 1. GNT's 1,2,4,5, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal
- 3. CMP-SA-PEG, ST3Gal3

a-m, r-y (independently selected) = 0 or 1; z = 1; n = 0; R = PEG.

FIG. 31F

Yeast expressed IF-beta a-n = 0; z = 1; r-y (independently selected) = 0 to 1; R (branched or linear) = Man, oligomannose.

- 1. mannosidases
- 2. GNT's 1,2,4,5, UDP-GlcNAc
- 3. Galactosyltransferase, UDP-Gal
- 4.. CMP-SA-PEG, ST3Gal3

a-m, r-y (independently selected) = 0 or 1; z=1; n=0; R=PEG.

FIG. 31G

```
NSO expressed IF-beta
a-i, r-u (independently selected) = 0 or 1;
j-m, n, v-y = 0; z = 1.
```

 CMP-SA-levulinate, ST3Gal3, buffer, salt
 2. H₄N₂-PEG

a-i, j-m, r-y (independently selected) = 0 or 1; n = 0; z = 1; R = PEG.

FIG. 31H

```
CHO, BHK, 293 cells, Vero expressed IF-beta h = 1 to 3; a-g, j-m, i (independently selected) = 0 or 1; r-u (independently selected) = 0 or 1; n, v-y = 0; z = 1.
```

1. CMP-SA-PEG, α2,8-ST

```
h = 1 to 3;
a-g, i, r-u (independently selected) = 0 or 1;
j-m (independently selected) = 0 to 2;
v-y (independently selected) = 1,
when j-m (independently selected) is 2;
z = 1; n = 0; R = PEG.
```

FIG. 311

CHO, BHK, 293 cells, Vero expressed IF-beta a-g, j-m, r-u (independently selected) = 0 or 1; h = 1 to 3; n, v-y = 0; z = 1.

- 1. Sialidase
- 2. Trans-sialidase, PEG-Sia-lactose

a-g, j-m, r-y (independently selected) = 0 or 1; h = 1 to 3; n = 0; z = 1; R = PEG.

FIG. 31J

CHO, BHK, 293 cells, Vero expressed Ifn-beta. a-d, i-m, r-u, z (independently selected) = 0 or 1; e-h=1; n, v-y=0.

- 1. Sialidase
- 2. CMP-SA-PEG (1.2 mol eq), ST3Gal3
- 3. CMP-SA (16 mol eq), ST3Gal3

a-d, i-m, r-u, z (independently selected) = 0 or 1; e-h = 1; n=0; v-y (independently selected) = 0 or 1; R = PEG.

FIG. 31K

```
NSO expressed Ifn-beta.

a-d, i-m, r-u, z (independently selected) = 0 or 1;

e-h = 1; n, v-y = 0;

Sia (independently selected) = Sia or Gal.
```

- 1. Sialidase and α -galactosidase
- 2. α-Galactosyltransferase, UDP-Gal

```
a-d, i-m, r-u, z (independently selected) = 0 or 1;
e-h = 1; R = PEG
n = 0; v-y (independently selected) = 1,
when j-m (independently selected) is 1;
```

FIG. 31L

```
CHO, BHK, 293 cells, Vero expressed Ifn-beta. a-d, i-m, r-u, z (independently selected) = 0 or 1; e-h=1; n, v-y=0.
```

- 1. Sialidase
- 2. CMP-SA-PEG (16 mol eq), ST3Gal3
- 3. CMP-SA, ST3Gal3

```
a-d, i-m, r-u, z (independently selected) = 0 or 1;
e-h = 1; n = 0;
v-y (independently selected) = 0 or 1; R = PEG.
```

FIG. 31M

CHO, BHK, 293 cells, Vero expressed Ifn-beta. a-d, i-m, r-u, z (independently selected) = 0 or 1; e-h=1; n, v-y=0.

 CMP-SA-levulinate, ST3Gal3, buffer, salt
 H₄N₂-PEG

a-d, i-m, r-u, z (independently selected) = 0 or 1; e-h = 1; n = 0; v-y (independently selected) = 0 or 1; R = PEG.

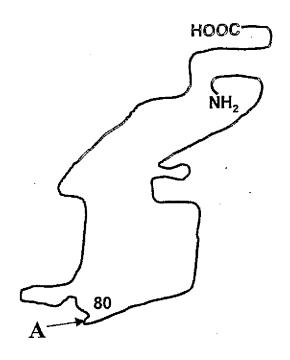
FIG. 31N

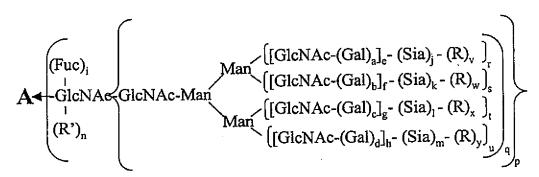
CHO, BHK, 293 cells, Vero expressed Ifn-beta. a-d, i-m, r-u, z (independently selected) = 0 or 1; e-h=1; n, v-y=0.

1. CMP-SA, α2,8-ST

a-d, i, r-u, z (independently selected) = 0 or 1; e-h = 1; j-m (independently selected) = 0-20; n, v-y (independently selected) = 0.

FIG. 310





a-d, i, p-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = modifying group; R' = H, glycosyl group, modifying group, glycoconjugate.

FIG. 31P

```
Insect cell expressed Ifn-beta.
a-d, f, h, j-m, s, u, v-y = 0;
e, g, i, q, r, t (independently selected) = 0 or 1.
```

- 1. GNT's 1,2,4,5, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal-PEG

```
a-i, q-u (independently selected) = 0 or 1;
j-m = 0; v-y (independently selected) = 1,
when e-h (independently selected) is 1;
R = PEG.
```

FIG. 31Q

```
Yeast expressed Ifn-beta.

a-m = 0; q-y (independently selected) = 0 to 1;

p = 1;

R (branched or linear) = Man, oligomannose.
```

- 1. Endoglycanase
- 2. Galactosyltransferase, UDP-Gal
- 3. CMP-SA-PEG, ST3Gal3

```
a-m, p-y = 0;
n (independently selected) = 0 or 1;
R' = -Gal-Sia-PEG.
```

FIG. 31R

```
CHO, BHK, 293 cells, Vero expressed Ifn-beta. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.
```

- 1. CMP-SA-linker-SA-CMP, ST3Gal3
- 2. ST3Gal3, desialylated transferrin.
- 3. CMP-SA, ST3Gal3

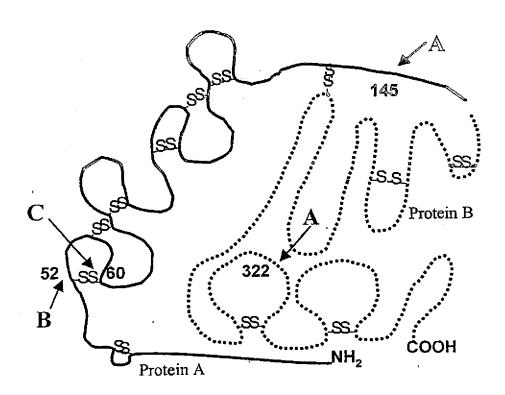
```
a-m, q-u (independently selected) = 0 or 1;

p = 1; n = 0;

v-y (independently selected) = 0 or 1;

R = linker-transferrin.
```

FIG. 31S



$$A \leftarrow \begin{bmatrix} (\operatorname{Fuc})_{i} & \operatorname{Man} \left[(\operatorname{GlcNAc-(Gal)}_{a})_{e} - (\operatorname{Sia})_{j} - (\operatorname{R})_{v} \right]_{r} \\ (\operatorname{GlcNAc-GlcNAc-Man} & \left[(\operatorname{GlcNAc-(Gal)}_{b})_{f} - (\operatorname{Sia})_{k} - (\operatorname{R})_{w} \right]_{s} \\ (\operatorname{GlcNAc-(Gal)}_{c})_{g} - (\operatorname{Sia})_{l} - (\operatorname{R})_{x} \right]_{t} \\ (\operatorname{GlcNAc-(Gal)}_{d})_{h} - (\operatorname{Sia})_{m} - (\operatorname{R})_{y} \right]_{u} = 0$$

$$\mathbf{B} \leftarrow \left\{ \text{Gic-}(Xyl)_n \right\}_0$$
 $\mathbf{C} \leftarrow \left\{ \text{Fuc } \right\}_p$

a-d, i, q-u (independently selected) = 0 or 1. o, p (independently selected) = 0 or 1. e-h, n (independently selected) = 0 to 6. j-m (independently selected) = 0 to 20. v-y=0; R = modifying group, mannose, oligomannose, Sia-Lewis X, Sia-Lewis A..

FIG. 32A

```
BHK expressed Factor VII or VIIa
a-d, e, i, g, q, j, l, o, p (independently selected) = 0 or 1;
r, t = 1; f, h, k, m, s, u, v-y = 0; n = 0-4.
```

- 1. Sialidase
- 2. CMP-SA-PEG (16 mole eq), ST3Gal3

```
a-d, e, g, i, q, j, l, o, p (independently selected) = 0 or 1;
r, t = 1; f, h, k, m, s, u, w, y = 0; n = 0-4;
v, x, (independently selected) = 1,
when j, l (respectively, independently selected) is 1;
R = PEG.
```

FIG. 32B

CHO, BHK, 293 cells, Vero expressed Factor VII or VIIa a-d, e, i, g, q, j, l, o, p (independently selected) = 0 or 1; r, t = 1; f, h, k, m, s, u, v-y = 0; n = 0-4.

- 1. Sialidase
- CMP-SA-PEG (1.2 mole eq), ST3Gal3
- 3. CMP-SA (8 mol eq), ST3Gal3

```
a-d, e, g, i, q, j, l, o, p (independently selected) = 0 or 1;
r, t = 1; f, h, k, m, s, u, w, y = 0; n = 0-4;
v or x, (independently selected) = 1,
when j or l, (respectively, independently selected) is 1;
R = PEG.
```

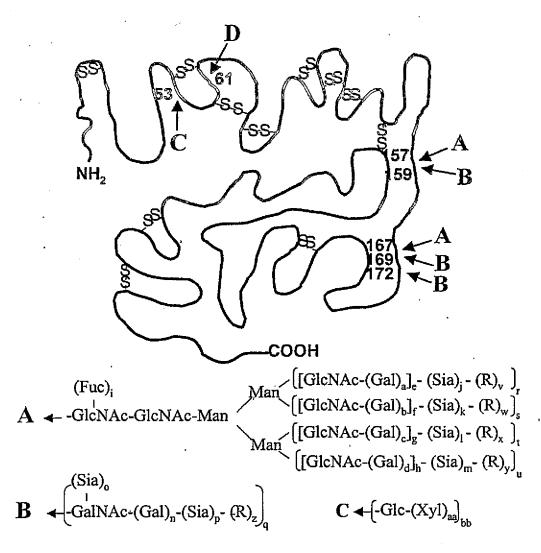
FIG. 32C

```
NSO expressed Factor VII or VIIa
a--u (independently selected) = 0 or 1;
v-y = 0; n = 0-4;
Sia (independently selected) = Sia or Gal.
```

- 1. Sialidase and α -galactosidase
- 2. Galactosyltransferase, UDP-Gal
- ★ 3. CMP-SA-PEG, ST3Gal3

```
a-m, o-u (independently selected) = 0 or 1;
n = 0-4; v-y (independently selected) = 1,
when j-m (independently selected) is 1;
Sia = Sia; R = PEG.
```

FIG. 32D



$$\mathbf{D} \longleftarrow \text{-Fuc} \Big\{ \text{-} (\text{GlcNAc})_{\text{cc}} \text{-} (\text{Gal})_{\text{dd}} \text{-} (\text{Sia})_{\text{ee}} \Big\}_{\text{ff}} \text{-} (R)_{\text{gg}}$$

a-d, i, n-u (independently selected) = 0 or 1.
bb, cc, dd, ee, ff, gg (independently selected) = 0 or 1.
e-h, aa (independently selected) = 0 to 6.
j-m (independently selected) = 0 to 20.
v-z = 0; R = modifying group, mannose, oligo-mannose.

FIG. 33A

```
CHO, BHK, 293 cells, Vero expressed Factor IX a-d, q = 1; e-h = 1 to 4; aa, bb, cc, dd, ee, ff, j-m, i, n, o, p, r-u (independently selected) = 0 or 1; v-z, gg = 0.
```

- 1. Sialidase
- 2. CMP-SA-PEG, ST3Gal3

```
a-d, q = 1; e-h = 1 to 4;
aa, bb, cc, dd, ee, ff, i, n, r-u (independently selected)
= 0 or 1;
o, p, z = 0;
j-m, ee, v-y, gg (independently selected) = 0 or 1;
R = PEG.
```

FIG. 33B

```
CHO, BHK, 293 cells, Vero expressed Factor IX
a-d, n, q = 1; e-h = 1 to 4;
aa, bb, cc, dd, ee, ff, j-m, i, o, p, r-u (independently selected) = 0 or 1;
v-z, gg = 0.
```

- 1. Sialidase
- 2. CMP-SA-PEG, ST3Gal3
- 3. ST3Gal1, CMP-SA

```
a-d, n, p, q = 1; e-h = 1 to 4;
aa, bb, cc, dd, ee, ff, i, r-u (independently selected) = 0 or 1;
j-m, ee, v-y, gg (independently selected) = 0 or 1;
o, z = 0; R = PEG.
```

FIG. 33C

CHO, BHK, 293 cells, Vero expressed Factor IX a-d, n, q, bb, cc, dd, ff = 1; e-h, aa = 1 to 4; ee, j-m, i, o, p, r-u (independently selected) = 0 or 1; v-z, gg = 0.

- 1. sialidase
- 2. Galactosyltransferase, UDP-Gal
- 3. CMP-SA, ST3Gal3
- 4. CMP-SA-PEG, ST3Gal1

```
a-d, n, q = 1; e-h = 1 to 4;
aa, bb, cc, dd, ee, ff, i, r-u (independently selected) =
0 or 1; R = PEG;
o, v-y, gg = 0;
j-m, p, ee (independently selected) = 0 or 1, but when
p = 1, z = 1.
```

FIG. 33D

```
CHO, BHK, 293 cells, Vero expressed Factor IX
a-d, q = 1; e-h = 1 to 4;
aa, bb, cc, dd, ee, ff, j-m, i, n, o, p, r-u (independently selected) = 0 or 1;
v-z, gg = 0.
```

CMP-SA-PEG, ST3Gal3

```
a-d, q = 1; e-h = 1 to 4;
aa, bb, cc, dd, ee, ff, i, n, r-u (independently selected)
= 0 or 1; R = PEG;
o, p, z = 0; j-m, ee, v-y, gg (independently selected) =
0 or 1.
```

FIG. 33E

```
CHO, BHK, 293 cells, Vero expressed Factor IX a-d, q = 1; e-h = 1 to 4; aa, bb, cc, dd, ee, ff, j-m, i, n, o, p, r-u (independently selected) = 0 or 1; v-z, gg = 0.
```

 CMP-SA-levulinate, ST3Gal3, buffer, salt
 H₄N₂-PEG

a-d, q = 1; e-h = 1 to 4; aa, bb, cc, dd, ee, ff, i, n, r-u (independently selected) = 0 or 1; o, p, z = 0; R = PEG; j-m, ee, v-y, gg (independently selected) = 0 or 1.

FIG. 33F

```
CHO, BHK, 293 cells, Vero expressed Factor IX
a-d, n, q, bb, cc, dd, ff = 1;
e-h, aa = 1 to 4;
ee, j-m, i, o, p, r-u (independently selected) = 0 or 1;
v-z, gg = 0.
```

1. CMP-SA-PEG, α 2,8-ST

```
a-d, q = 1; e-h = 1 to 4;

aa, bb, cc, dd, ee, ff, i, n, r-u (independently selected)

= 0 or 1;

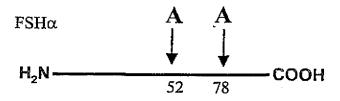
o, p, z = 0; R= PEG;

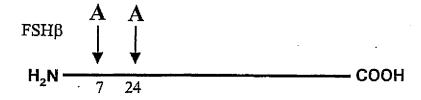
j-m, ee (independently selected) = 0 to 2;

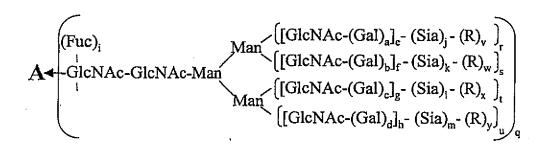
v-y, gg (independently selected) = 1, when j-m

(independently selected) is 2;
```

FIG. 33G







a-d, i, q-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = modifying group, mannose, oligo-mannose.

FIG. 34A

```
CHO, BHK, 293 cells, Vero expressed FSH. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.
```

- 1. Sialidase
- 2. CMP-SA-PEG (16 mol eq), ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 1,
when j-m (independently selected) is 1;
R = PEG.
```

FIG. 34B

```
CHO, BHK, 293 cells, Vero expressed FSH. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.
```

- 1. Sialidase
- 2. CMP-SA-PEG (1.2 mol eq), ST3Gal3
- 3. CMP-SA (16 mol eq), ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.
```

FIG. 34C

```
NSO expressed FSH.

a-d, i-m, q-u (independently selected) = 0 or 1;

e-h = 1; v-y = 0;

Sia (independently selected) = Sia or Gal.
```

- 1. Sialidase and α-galactosidase
- 2. Galactosyltransferase, UDP-Gal
- ▼ 3. CMP-SA-PEG, ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y (independently selected) = 1, when j-m (independently selected) is 1; R = PEG.

FIG. 34D

```
CHO, BHK, 293 cells, Vero expressed FSH. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.
```

- 1. Sialidase
- CMP-SA-PEG (16 mol eq), ST3Gal3
- 3. CMP-SA, ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y (independently selected) = 0 or 1; R = PEG.

FIG. 34E

CHO, BHK, 293 cells, Vero expressed FSH. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.

 CMP-SA-levulinate, ST3Gal3, buffer, salt
 H₄N₂-PEG

a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y (independently selected) = 0 or 1; R = PEG.

FIG. 34F

CHO, BHK, 293 cells, Vero expressed FSH. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.

1. CMP-SA, α2,8-ST

a-d, i, q-u (independently selected) = 0 or 1; e-h = 1; j-m (independently selected) = 0-20; v-y (independently selected) = 0.

FIG. 34G

```
Insect cell expressed FSH.
a-d, f, h, j-m, s, u, v-y = 0;
e, g, i, q, r, t (independently selected) = 0 or 1.
```

- 1. GNT's 1,2,4,5, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal-PEG

```
a-i, q-u (independently selected) = 0 or 1;
j-m = 0; v-y (independently selected) = 1,
when e-h (independently selected) is 1;
R = PEG.
```

FIG. 34H

```
Yeast expressed FSH.

a-m = 0; q-y (independently selected) = 0 to 1;

p = 1;

R (branched or linear) = Man, oligomannose.
```

- 1. Endoglycanase
- 2. Galactosyltransferase, UDP-Gal
- ▼ 3. CMP-SA-PEG, ST3Gal3

```
a-m, p-y = 0;
n (independently selected) = 0 or 1;
R' = -Gal-Sia-PEG.
```

FIG. 341

```
CHO, BHK, 293 cells, Vero expressed FSH. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.
```

- 1. CMP-SA-linker-SA-CMP, ST3Gal3
- 2. ST3Gal1, desialylated chorionic gonadrophin (CG) produced in CHO.
- 3. CMP-SA, ST3Gal3, ST3Gal1

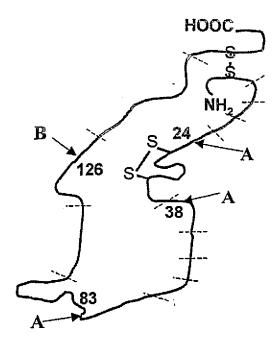
```
a-m, q-u (independently selected) = 0 or 1;

p = 1; n = 0;

v-y (independently selected) = 0 or 1;

R = linker-CG.
```

FIG. 34J



$$(Fuc)_{i} \qquad Man \begin{cases} [GlcNAc-(Gal)_{a}]_{e} - (Sia)_{j} - (R)_{v} \end{cases}_{r} \\ A \leftarrow -GlcNAc-GlcNAc-Man \qquad Man \begin{cases} [GlcNAc-(Gal)_{b}]_{f} - (Sia)_{k} - (R)_{w} \end{cases}_{s} \\ Man \begin{cases} [GlcNAc-(Gal)_{b}]_{g} - (Sia)_{l} - (R)_{x} \end{cases}_{t} \\ [GlcNAc-(Gal)_{d}]_{h} - (Sia)_{m} - (R)_{y} \end{cases}_{u} \\ B \leftarrow \begin{pmatrix} Sia)_{o} \\ -GalNAc-(Gal)_{n} - (Sia)_{p} - (R)_{z} \end{pmatrix}_{q} \end{cases}$$

a-d, i, n-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 4. j-m (independently selected) = 0 to 20. v-z = 0; R = polymer.

FIG. 35A

```
CHO, BHK, 293 cells, Vero expressed EPO a-g, n, q = 1; h = 1 to 3; j-m, i, o, p (independently selected) = 0 or 1; r-u (independently selected) = 0 to 1; v-z = 0
```

- 1. Sialidase
- 2. CMP-SA-PEG, ST3Gal3

```
a-g, n, q = 1; h = 1 to 3;

i, o, p (independently selected) = 0 or 1;

r-u (independently selected) = 0 or 1;

j-m, v-y (independently selected) = 0 or 1;

R = PEG; z = 0.
```

FIG. 35B

```
Insect cell expressed EPO
a-d, f, h, j-q, s, u, v-z = 0;
e, g, i, r, t (independently selected) = 0 or 1.
```

- 1. GNT's 1&2, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal
- 2. CMP-SA-PEG, ST3Gal3

```
b, d, f, h, k, m-q, s, u, w, y, z = 0;
a, c, e, g, i, r, t (independently selected)= 0 or 1;
j, l, v, x (independently selected) = 0 or 1;
R = PEG.
```

FIG. 35C

```
CHO, BHK, 293 cells, Vero expressed EPO a-q, r-u (independently selected) = 0 or 1; v-z = 0.
```

- 1. sialidase
- 2. Galactosyltransferase, UDP-Gal
- 3. CMP-SA, ST3Gal3
- 4. CMP-SA-PEG, ST3Gall

```
a-h, n, q = 1;
i-m, o, r-u (independently selected) = 0 or 1;
v-y = 0; p, z = 0 or 1; R = PEG.
```

FIG. 35D

```
CHO, BHK, 293 cells, Vero expressed EPO a-g, n, q = 1; h = 1 to 3; j-m, i, o, p (independently selected) = 0 or 1; r-u (independently selected) = 0 or 1; v-z = 0
```

1. CMP-SA-PEG, ST3Gal3

```
a-g, n, q = 1; h = 1 to 3;
i, o, p (independently selected) = 0 or 1;
r-u (independently selected) = 0 to 1;
j-m, v-y (independently selected) = 0 or 1;
R = PEG; z = 0.
```

FIG. 35E

Insect cell, yeast or fungi expressed EPO a-d, f, h, j-q, s, u, v-z=0; e, g, i, r, t (independently selected) = 0 or 1.

- 1. GNT's 1, 2 & 5, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal-PEG

a-c, e-g, i, r-t, v-x (independently selected) = 0 or 1; d, h, j-q, u, y, z = 0; R = PEG.

FIG. 35F

Insect cell, yeast or fungi expressed EPO a-d, f, h, j-q, s, u, v-z = 0; e, g, i, r, t (independently selected) = 0 or 1.

- 1. GNT's 1, 2 & 5, UDP-GlcNAc
- 2. Galactosidase (synthetic enzyme), PEG-Gal-F.

a-c, e-g, n, q-t, v-x, z (independently selected) = 0 or 1; d, h, j-m, o, p, y, z = 0; R = PEG.

FIG. 35G

Insect cell, yeast or fungi expressed EPO a-d, f, h, j-m, n-q, s, u, v-z = 0; e, g, i, r, t (independently selected) = 0 or 1.

1. GNT-1, UDP-GlcNAc-PEG

e, i, r, v (independently selected) = 0 or 1; a-h, j-q, s-u, w-z = 0; R = PEG.

FIG. 35H

Insect cell, yeast or fungi expressed EPO a-d, f, h, j-m, n-q, s, u, v-z = 0; e, g, i, r, t (independently selected) = 0 or 1.

- 1. GNT-1, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal-PEG

a, e, i, r, v (independently selected) = 0 or 1; b-d, f-h, j-q, s-u, w-z = 0; R = PEG.

FIG. 351

Insect cell, yeast or fungi expressed EPO a-d, f, h, j-m, n-q, s, u, v-z = 0; e, g, i, r, t (independently selected) = 0 or 1.

- 1. GNT-1, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal
- 3. ST3Gal3, CMP-SA-PEG

a, e, i, j, r, v (independently selected) = 0 or 1; b-d, f-h, k-q, s-u, w-z = 0; R = PEG.

FIG. 35J

Insect cell, yeast or fungi expressed EPO a-d, f, h, j-m, n-q, s, u, v-z = 0; e, g, i, r, t (independently selected) = 0 or 1.

- 1. GNT's 1, 2 & 5, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal
- 3. ST3Gal3, CMP-SA-PEG

a-c, e-g, i-l, r-t, v-x (independently selected)
= 0 or 1;
d, h, m-q, u, y, z = 0; R = PEG.

FIG. 35K

```
Insect cell, yeast or fungi expressed EPO a-d, f, h, j-m, n-q, s, u, v-z = 0; e, g, i, r, t (independently selected) = 0 or 1.
```

- 1. GNT's 1, 2 & 5, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal
- ▼ 3. α2,6-sialyltransferase, CMP-SA-PEG

```
a-c, e-g, i-l, r-t, v-x (independently selected)
= 0 or 1;
d, h, m-q, u, y, z = 0; R = PEG.
```

FIG. 35L

```
CHO, BHK, 293 cells, Vero expressed EPO
a-q, r-u (independently selected) = 0 or 1;
v-z = 0.

1. sialidase
2. CMP-SA, ST3Gal3
3. CMP-SA-PEG, ST3Gal1

a-h, q, i-o, r-u (independently selected)
= 0 or 1;
v-y = 0; p, z = 0 or 1; R = PEG.
```

FIG. 35M

CHO, BHK, 293 cells, Vero expressed EPO a-q, r-u (independently selected) = 0 or 1; v-z = 0.

1. CMP-SA-PEG, ST3Gal3

a-h, i-o, q-u (independently selected) = 0 or 1; v-y = 0; p, z = 0 or 1; R = PEG.

FIG. 35N

CHO, BHK, 293 cells, Vero expressed EPO a-q, r-u (independently selected) = 0 or 1; v-z = 0.

1. CMP-SA-PEG, α 2,8-sialyltransferase

a-h, i-o, q-u (independently selected) = 0 or 1; v-y = 0; p, z = 0 or 1; R = SA-PEG.

FIG. 350

CHO, BHK, 293 cells, Vero expressed EPO a-q, r-u (independently selected) = 0 or 1; v-z = 0.

1. CMP-SA-PEG, α2,8-sialyltransferase

a-h, i-o, p-u, v-z (independently selected) = 0 or 1; R = SA-PEG.

FIG. 35P

yeast or fungi expressed EPO
r, t, u, v, x, y (independently selected) = 0 or 1;
a-m, n-q, s, w, z = 0; R = (Man)_n
where n = 1-5, linear or branched.

- 1. mannosidases
- 2. GNT-1, UDP-GlcNAc
- 3. galactosyltransferase, UDP-Gal
- 4. ST3Gal3, CMP-SA-PEG

a, e, j, r, v (independently selected) = 0 or 1; b-d, f-i, k-q, s-u, w-z = 0; R = PEG.

FIG. 35Q

```
yeast or fungi expressed EPO
r, t, u, v, x, y (independently selected) = 0 or 1;
a-m, n-q, s, w, z = 0; R = (Man)<sub>n</sub>
where n = 1-5, linear or branched.
```

- 1. mannosidases
- 2. GNT-1, UDP-GlcNAc-PEG

e, r, v (independently selected) = 0 or 1; a-h, i-q, s-u, w-z = 0; R = PEG.

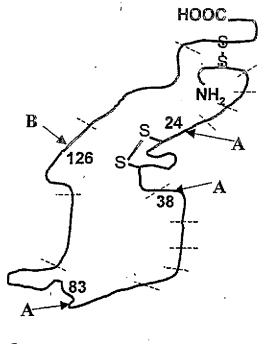
FIG. 35R

yeast or fungi expressed EPO r, t, u, v, x, y (independently selected) = 0 or 1; a-m, n-q, s, w, z = 0; $R = (Man)_n$ where n = 1-5, linear or branched.

- 1. mannosidase-I
- 2. GNT-1, UDP-GlcNAc
- 3. galactosyltransferase, UDP-Gal
- 4. ST3Gal3, CMP-SA-PEG

```
a, e, j, r, t-u, v, x, y (independently selected)
= 0 or 1;
b-d, f-i, k-q, s, w, z = 0;
(R)<sub>v</sub> = PEG; (R)<sub>x</sub> and (R)<sub>y</sub> = Man.
```

FIG. 35S



$$\mathbf{A} \leftarrow \text{-GlcNAc-}(Gal)_{a}l_{e} - (Sia)_{j} - (R)_{v} \\ \mathbf{A} \leftarrow \text{-GlcNAc-}(Gal)_{b}l_{f} - (Sia)_{k} - (R)_{w} \\ \mathbf{A} \leftarrow \text{-GlcNAc-}(Gal)_{b}l_{f} - (Sia)_{h} - (R)_{w} \\ \mathbf{A} \leftarrow \text{-GlcNAc-}(Gal)_{h}l_{h} - (Sia)_{h}l_{h} - (Sia)_{h}l_{h} - (Sia)_{h}l_{h} \\ \mathbf{A} \leftarrow \text{-GlcNAc-}(Gal)_{h}l_{h} - (Sia)_{h}l_{h} - (Sia)_{h}l_{h} \\ \mathbf{A} \leftarrow \text{-GlcNAc-}(Gal)_{h}l_{h} \\ \mathbf{A} \leftarrow$$

a-d, i, n-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 4. j-m (independently selected) = 0 to 20. v-z = 0; aa, bb = 1; cc = 0;

R = polymer; R'' and R' = sugar-polymer or Fuc.

FIG. 35T

```
yeast or fungi expressed EPO
r, t, u, v, x, y (independently selected) = 0 or 1;
cc, a-m, n-q, s, w, z = 0;
aa, bb = 1;
R = (Man), where n = 1-100, linear or branched.
```

- 1. endo-H
- 2. galactosyltransferase, UDP-Gal-PEG

```
i (independently selected) = 0 or 1;
aa, bb, cc, a-h, j-z = 0; R" = Gal-PEG.
```

FIG. 35U

```
yeast or fungi expressed EPO
r, t, u, v, x, y (independently selected) = 0 or 1;
cc, a-m, n-q, s, w, z = 0; aa, bb = 1;
R = (Man)<sub>n</sub> where n = 1-100, linear or branched.
```

- 1. endo-H
- 2. galactosyltransferase, UDP-Gal
- 3. ST3Gal3, CMP-SA-PEG

```
i (independently selected) = 0 or 1;
aa, bb, cc, a-h, j-z = 0; R" = Gal-SA-PEG.
```

FIG. 35V

```
Insect cell expressed EPO

a-d, f, h, j-m, n-q, s, u, v-z = 0;

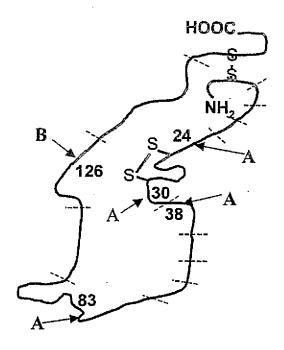
e, g, i, r, t (independently selected) = 0 or 1;

aa = 1; R" = Fuc.
```

- 1. mannosidases
- 2. galactosyltransferase, UDP-Gal-PEG

```
cc, e, i, r, v (independently selected) = 0 or 1;
bb, a-h, j-q, s-u, w-z = 0; aa = 1; R' = Gal-PEG.
```

FIG. 35W



$$(Fuc)_{i}$$

$$A \leftarrow -GlcNAc-GlcNAc-Man$$

$$= -GlcNAc-GlcNAc-GlcNAc-GlcNac-Gll)t - (Sia)t - (Sia$$

FIG. 35X

```
NSO expressed NESP
q = 1; a-i, n, r-u (independently selected) = 0
or 1; j-m, o, p, v-z = 0
```

 CMP-SA-levulinate, ST3Gal3, buffer, salt
 H_aN₂-PEG

q = 1; a-i, j-n, r-y (independently selected) = 0 or 1;
o, p, z = 0; R = PEG.

FIG. 35Y

```
CHO, BHK, 293 cells, Vero expressed NESP a-g, n, q = 1; h = 1 to 3; j-m, i, o, p (independently selected) = 0 or 1; r-u (independently selected) = 0 or 1; v-z = 0
```

1. CMP-SA-PEG, α 2,8-ST

```
a-g, n, q = 1; h = 1 to 3;

i, o, p (independently selected) = 0 or 1;

r-u (independently selected) = 0 to 1;

j-m (independently selected) = 0 to 2;

v-y (independently selected) = 1,

when j-m (independently selected) is 2;

R = PEG; z = 0.
```

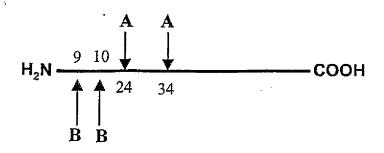
FIG. 35Z

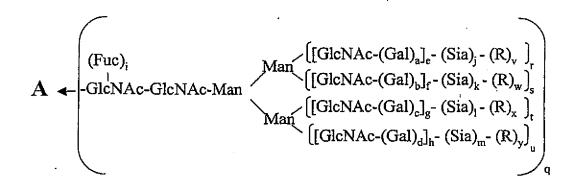
CHO, BHK, 293 cells, Vero expressed NESP a-g, n, q = 1; h = 1 to 3; j-m, i, o, p (independently selected) = 0 or 1; r-u (independently selected) = 0 to 1; v-z = 0

1 CMP-SA, poly- α 2,8-ST

a-g, n, q = 1; h = 1 to 3; i, j-m, o, p, r-u, (independently selected) = 0 or 1; v-z (independently selected) = 0-40; R = Sia.

FIG. 35AA





$$\mathbf{B} \leftarrow \begin{pmatrix} (\operatorname{Sia})_{o} \\ -\operatorname{GalNAc-(Gal)}_{n} - (\operatorname{Sia})_{p} - (\operatorname{R})_{z} \end{pmatrix}_{aa}$$

a-d, i, n-u, aa (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = polymer, glycoconjugate.

FIG. 36A

CHO, BHK, 293 cells, Vero expressed GM-CSF. a-d, i-m, o-u, aa (independently selected) = 0 or 1; n, e-h = 1; v-z = 0.

- 1. Sialidase
- 2. CMP-SA-PEG (16 mol eq), ST3Gal3

a-d, i-m, q-u, aa (independently selected) = 0 or 1;
o, p, z = 0; n, e-h = 1;
v-y (independently selected) = 1,
when j-m (independently selected) is 1;
R = PEG.

FIG. 36B

CHO, BHK, 293 cells, Vero expressed GM-CSF. a-d, i-m, o-u, aa (independently selected) = 0 or 1; n, e-h = 1; v-z = 0.

- 1. Sialidase
- 2. CMP-SA-PEG (1.2 mol eq), ST3Gal3
- 3. CMP-SA (16 mol eq), ST3Gal3 & ST3Gal1

a-d, i-m, p-u, aa (independently selected) = 0 or 1; o, z = 0; n, e-h = 1; v-y (independently selected) = 0 or 1; R = PEG.

FIG. 36C

```
NSO expressed GM-CSF.
a-d, i-m, o-u, aa (independently selected) = 0 or 1;
n, e-h = 1; v-z = 0;
Sia (independently selected) = Sia or Gal.
```

- 1. Sialidase and α-galactosidase
- 2. CMP-SA, ST3Gal3
- 2. CMP-SA-PEG, ST3Gal1

a-d, i-m, p-u, z, aa (independently selected) = 0 or 1; n, e-h = 1; o, v-y = 0; z = 1, when p = 1; R = PEG.

FIG. 36D

```
CHO, BHK, 293 cells, Vero expressed GM-CSF. a-d, i-m, o-u, aa (independently selected) = 0 or 1; n, e-h = 1; v-z = 0.
```

- 1. Sialidase
- 2. CMP-SA-PEG (16 mol eq), ST3Gal3
- 3. CMP-SA, ST3Gal3

a-d, i-m, q-y, as (independently selected) = 0 or 1; o, p, z = 0; n, e-h = 1; R = PEG.

FIG. 36E

CHO, BHK, 293 cells, Vero expressed GM-CSF. a-d, i-m, o-u, aa (independently selected) = 0 or 1; n, e-h=1; v-z=0.

- 1. CMP-SA-levulinate, ST3Gal3, buffer, salt
- 2. H₄N₂-PEG

a-d, i-m, o-y, aa (independently selected) = 0 or 1; z = 0; n, e-h = 1; R = PEG.

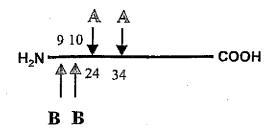
FIG. 36F

CHO, BHK, 293 cells, Vero expressed GMCSF. a-d, i-m, o-u, aa (independently selected) = 0 or 1; n, e-h=1; v-z=0.

1. CMP-SA, α2,8-ST

a-d, i, o-u, aa (independently selected) = 0 or 1; n, e-h = 1; j-m (independently selected) = 0-20; v-z (independently selected) = 0.

FIG. 36G



$$A \leftarrow \begin{array}{c} \\ \text{(Fuc)}_{i} \\ \text{GlcNAc-(Gal)}_{a}]_{e} - (\text{Sia)}_{j} - (\text{R)}_{v} \\ \text{[GlcNAc-(Gal)}_{b}]_{f} - (\text{Sia)}_{k} - (\text{R)}_{w} \\ \text{[GlcNAc-(Gal)}_{c}]_{g} - (\text{Sia)}_{1} - (\text{R)}_{x} \\ \text{(R')}_{cc} \\ \end{array}$$

$$\begin{array}{c} \text{(GlcNAc-(Gal)}_{b}]_{f} - (\text{Sia)}_{g} - (\text{Sia)}_{1} - (\text{R)}_{x} \\ \text{[GlcNAc-(Gal)}_{d}]_{h} - (\text{Sia)}_{m} - (\text{R)}_{y} \\ \text{(b)} \\ \end{array}$$

$$\mathbf{B} \leftarrow \begin{bmatrix} (\mathrm{Sia})_{0} \\ -\mathrm{GalNAc} \cdot (\mathrm{Gal})_{n} \cdot (\mathrm{Sia})_{p} \cdot (\mathrm{R})_{z} \end{bmatrix}_{\mathrm{aa}}$$

a-d, i, n-u, aa, bb, cc (independently selected) = 0 or 1.
e-h (independently selected) = 0 to 6.
j-m (independently selected) = 0 to 100.
v-y = 0; R = modifying group, mannose, oligo-mannose.
R'= H, glycosyl residue, modifying group. glycoconjugate.

FIG. 36H

```
Insect cell expressed GM-CSF.
a-d, f, h, j-m, o, p, s, u, v-z = 0;
e, g, i, n, q, r, t, aa (independently selected) = 0 or 1.
```

- 1. GNT's 1,2,4,5, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal-PEG

```
a-i, n, q-u (independently selected) = 0 or 1;

j-m = 0; v-y (independently selected) = 1,

when e-h (independently selected) is 1;

R = PEG.
```

FIG. 361

```
Yeast expressed GM-CSF.
a-p, z, cc = 0;
q-y, aa (independently selected) = 0 to 1;
bb = 1; R (branched or linear) = Man, oligomannose;
GalNAc = Man.
```

- 1. Endoglycanase
- 2. mannosidase (if aa = 1).
- 3. Galactosyltransferase, UDP-Gal-PEG

```
a-p, r-z, aa, bb = 0;
q, cc (independently selected) = 0 or 1;
R' = -Gal-PEG.
```

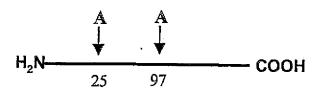
FIG. 36J

CHO, BHK, 293 cells, Vero expressed GM-CSF. a-m, o-u, aa, bb (independently selected) = 0 or 1; n, v-z, cc = 0.

- 1. sialidase
- 2. CMP-SA, ST3Gal3
- 2. CMP-SA-linker-SA-CMP, ST3Gal1
- 3. ST3Gal3, transferrin

a--m, p-u, z, as (independently selected) = 0 or 1; o, v-y, cc = 0; bb, n = 1; R = transferrin.

FIG. 36K



$$\mathbf{A} \leftarrow \begin{bmatrix} \left[\operatorname{GlcNAc-(Gal)}_{a} \right]_{e^{-}} \left(\operatorname{Sia} \right)_{j} - \left(\operatorname{R} \right)_{v} \end{bmatrix}_{r} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{b} \right]_{f^{-}} \left(\operatorname{Sia} \right)_{k} - \left(\operatorname{R} \right)_{w} \right]_{s} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{c} \right]_{g^{-}} \left(\operatorname{Sia} \right)_{l} - \left(\operatorname{R} \right)_{x} \right]_{t} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left(\operatorname{Sia} \right)_{m^{-}} \left(\operatorname{R} \right)_{y} \right]_{u} \end{bmatrix}_{t} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left(\operatorname{Sia} \right)_{m^{-}} \left(\operatorname{R} \right)_{y} \right]_{u} \right]_{t} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left(\operatorname{Sia} \right)_{m^{-}} \left(\operatorname{R} \right)_{y} \right]_{u} \right]_{t} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left(\operatorname{Sia} \right)_{m^{-}} \left(\operatorname{R} \right)_{y} \right]_{u} \right]_{t} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left(\operatorname{Sia} \right)_{m^{-}} \left(\operatorname{R} \right)_{y} \right]_{u} \right]_{t} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left(\operatorname{Sia} \right)_{m^{-}} \left(\operatorname{R} \right)_{y} \right]_{u} \right]_{t} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left(\operatorname{Sia} \right)_{m^{-}} \left(\operatorname{R} \right)_{y} \right]_{u} \right]_{t} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left(\operatorname{Sia} \right)_{m^{-}} \left(\operatorname{R} \right)_{y} \right]_{u} \right]_{t} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left(\operatorname{Sia} \right)_{m^{-}} \left(\operatorname{R} \right)_{y} \right]_{u} \right]_{t} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left(\operatorname{Sia} \right)_{m^{-}} \left(\operatorname{R} \right)_{y} \right]_{u} \right]_{t} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left(\operatorname{Sia} \right)_{m^{-}} \left(\operatorname{R} \right)_{y} \right]_{u} \right]_{t} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left(\operatorname{Sia} \right)_{m^{-}} \left(\operatorname{R} \right)_{y} \right]_{u} \right]_{t} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left(\operatorname{Sia} \right)_{m^{-}} \left(\operatorname{R} \right)_{y} \right]_{u} \right]_{t} \\ \left[\operatorname{GlcNAc-(Gal)}_{d^{-}} \left(\operatorname{Sia} \right)_{m^{-}} \left(\operatorname{R} \right)_{y} \right]_{t} \\ \left[\operatorname{GlcNAc-(Gal)}_{d^{-}} \left(\operatorname{Sia} \right)_{m^{-}} \left(\operatorname{R} \right)_{y} \right]_{t} \\ \left[\operatorname{GlcNAc-(Gal)}_{d^{-}} \left(\operatorname{Sia} \right)_{m^{-}} \left(\operatorname{R} \right)_{y} \right]_{t} \\ \left[\operatorname{GlcNAc-(Gal)}_{d^{-}} \left(\operatorname{Sia} \right)_{m^{-}} \left(\operatorname{R} \right)_{y} \right]_{t} \\ \left[\operatorname{GlcNAc-(Gal)}_{d^{-}} \left(\operatorname{Sia} \right)_{m^{-}} \left(\operatorname{R} \right)_{t} \right]_{t} \\ \left[\operatorname{GlcNAc-(Gal)}_{d^{-}} \left(\operatorname{Sia} \right)_{m^{-}} \left(\operatorname{R} \right)_{t} \right]_{t} \\ \left[\operatorname{GlcNAc-(Gal)}_{d^{-}} \left(\operatorname{Sia} \right)_{m^{-}} \left(\operatorname{R} \right)_{t} \right]_{t} \\ \left[\operatorname{GlcNAc-(Gal)}_{d^{-}} \left(\operatorname{Sia} \right)_{m^{-}} \left(\operatorname{Sia} \right)_{t} \right]_{t} \\ \left[\operatorname{GlcNAc-(Gal)}_{d^{-}} \left(\operatorname{Sia} \right)_{t} \right]_{t} \\ \left[\operatorname{GlcNAc-$$

a-d, i, q-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = polymer.

FIG. 37A

```
CHO, BHK, 293 cells, Vero expressed IF-gamma. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.
```

- 1. Sialidase
- 2. CMP-SA-PEG (16 mol eq), ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 1,
when j-m (independently selected) is 1;
R = PEG.
```

FIG. 37B

```
CHO, BHK, 293 cells, Vero expressed IF-gamma. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.
```

- 1. Sialidase
- 2. CMP-SA-PEG (1.2 mol eq), ST3Gal3
- 3. CMP-SA (16 mol eq), ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.
```

FIG. 37C

```
NSO expressed Interferon gamma.

a-d, i-m, q-u (independently selected) = 0 or 1;

e-h = 1; v-y = 0;

Sia (independently selected) = Sia or Gal.
```

- 1. Sialidase and α-galactosidase
- 2. α-Galactosyltransferase, UDP-Gal

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 1,
when j-m (independently selected) is 1;
R = PEG.
```

FIG. 37D

```
CHO, BHK, 293 cells, Vero expressed
Interferon gamma.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.
```

- 1. Sialidase
- 2. CMP-SA-PEG (16 mol eq), ST3Gal3
- 3. CMP-SA, ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.
```

FIG. 37E

```
CHO, BHK, 293 cells, Vero expressed
Interferon gamma.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.
```

```
1. CMP-SA-levulinate, ST3Ga13,
```

2. H_4N_2 -PEG

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.
```

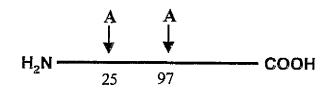
FIG. 37F

```
CHO, BHK, 293 cells, Vero expressed
Interferon gamma.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.
```

1. CMP-SA, α2,8-ST

```
a-d, i, q-u (independently selected) = 0 or 1;
e-h = 1; j-m (independently selected) = 0-20;
v-y (independently selected) = 0.
```

FIG. 37G



$$A = (\operatorname{Fuc})_{i} \\ \operatorname{GlcNAc-\operatorname{Gal})_{a}}_{e} - (\operatorname{Sia})_{j} - (\operatorname{R})_{v} \\ \operatorname{GlcNAc-\operatorname{Gal})_{b}}_{r} - (\operatorname{Sia})_{k} - (\operatorname{R})_{w} \\ \operatorname{GlcNAc-\operatorname{Gal})_{b}}_{r} - (\operatorname{Sia})_{k} - (\operatorname{R})_{w} \\ \operatorname{GlcNAc-\operatorname{Gal})_{c}}_{g} - (\operatorname{Sia})_{l} - (\operatorname{R})_{x} \\ \operatorname{[GlcNAc-\operatorname{Gal})_{d}}_{h} - (\operatorname{Sia})_{m} - (\operatorname{R})_{y} \\ \operatorname{[GlcNAc-\operatorname{Gal})_{d}}_{p} - (\operatorname{Sia})_{m} - (\operatorname{R})_{m} - (\operatorname{R})_{m} - (\operatorname{R})_{m} \\ \operatorname{[GlcNAc-\operatorname{Gal})_{d}}_{p} - (\operatorname{Sia})_{m} - (\operatorname{R})_{m} - (\operatorname{R})_{m} - (\operatorname{R})_{m} \\ \operatorname{[GlcNAc-\operatorname{Gal})_{d}}_{p} - (\operatorname{Sia})_{m} - (\operatorname{R})_{m} - (\operatorname{R})_{m}$$

a-d, i, n, p-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = modifying group, mannose, oligo-mannose; R' = H, glycosyl residue, modifying group, glycoconjugate.

FIG. 37H

```
Insect or fungi cell expressed IF-gamma.
a-d, f, h, j-m, s, u, v-y = 0;
e, g, i, q, r, t (independently selected) = 0 or 1.
```

- 1. GNT's 1,2,4,5, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal-PEG

```
a-i, q-u (independently selected) = 0 or 1;
j-m = 0; v-y (independently selected) = 1,
when e-h (independently selected) is 1;
R = PEG.
```

FIG. 371

```
Yeast expressed IF-gamma.

a-m = 0; q-y (independently selected) = 0 to 1; p = 1;

R (branched or linear) = Man, oligomannose.
```

- 1. Endoglycanase
- 2. Galactosyltransferase, UDP-Gal
- 3. CMP-SA-PEG, ST3Gal3

```
a-m, p-y = 0; n (independently selected) = 0 or 1; R' = -Gal-Sia-PEG.
```

FIG. 37J

```
CHO, BHK, 293 cells, Vero expressed IF-gamma. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.
```

- 1. CMP-SA-linker-Gal-UDP, ST3Gal3
- 2. Galactosyltransferase, transferrin treated with endoglycanase.

```
a-m, q-u (independently selected) = 0 or 1;

p = 1; n = 0;

v-y (independently selected) = 0 or 1;

R = linker-transferrin.
```

FIG. 37K

```
CHO, BHK, 293 cells, Vero expressed
Interferon gamma.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h, p = 1; n, v-y = 0.
```

1. CMP-SA-PEG, ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h, p = 1;
n, v-y (independently selected) = 0 or 1;
R = PEG.
```

FIG. 37L

```
a-d, f, h, j-n, s, u, v-y = 0;
e, g, i, q, r, t (independently selected) = 0 or 1.

1. GNT's 1 & 2, UDP-GlcNAc-PEG

a-d, f, h, j-n, s, u, w, y = 0;
e, g, i, r, t, q (independently selected) = 0 or 1;
p = 1; v, x (independently selected) = 1,
when e, g (independently selected) is 1;
R = PEG.
```

Insect or fungi cell expressed IF-gamma.

FIG. 37M

```
CHO, BHK, 293 cells, Vero expressed
Interferon gamma.

a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.

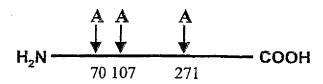
1. CMP-SA-PEG, α2,8-ST

a-d, i, q-u (independently selected) = 0 or 1;
e-h = 1; j-m (independently selected) = 0-2;
v-y (independently selected) = 1,
```

when j-m (independently selected) = 2;

FIG. 37N

R = PEG.



$$\mathbf{A} \leftarrow \begin{bmatrix} (\operatorname{Fuc})_{i} & & & \\ (\operatorname{Fuc})_{i} & & & \\ (\operatorname{GlcNAc-(Gal)}_{a}]_{e^{-}} (\operatorname{Sia})_{j} - (\operatorname{R})_{v} \end{bmatrix}_{r} \\ (\operatorname{GlcNAc-GlcNAc-Man} & & & \\ (\operatorname{GlcNAc-(Gal)}_{b}]_{f^{-}} (\operatorname{Sia})_{k} - (\operatorname{R})_{w} \end{bmatrix}_{s} \\ (\operatorname{GlcNAc-(Gal)}_{c}]_{g^{-}} (\operatorname{Sia})_{l} - (\operatorname{R})_{x} \end{bmatrix}_{t} \\ (\operatorname{GlcNAc-(Gal)}_{d}]_{h^{-}} (\operatorname{Sia})_{m^{-}} (\operatorname{R})_{y} \end{bmatrix}_{u}$$

a-d, i, q-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = polymer.

FIG. 38A

```
CHO, BHK, 293 cells, Vero or transgenic animal expressed α<sub>1</sub> antitrypsin.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.
```

- 1. Sialidase
- 2. CMP-SA-PEG (16 mol eq), ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 1,
when j-m (independently selected) is 1;
R = PEG.
```

FIG. 38B

```
CHO, BHK, 293 cells, Vero or transgenic animal expressed α<sub>1</sub> antitrypsin.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.
```

- 1. Sialidase
- CMP-SA-PEG (1.2 mol eq), ST3Gal3
- 3. CMP-SA (16 mol eq), ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.
```

FIG. 38C

```
CHO, BHK, 293 cells, Vero or transgenic animal expressed alpha-1 antitrypsin.

a-d, i-m, q-u (independently selected) = 0 or 1;

e-h = 1; v-y = 0.
```

- 1. Sialidase
- 2. CMP-SA-PEG (16 mol eq), ST3Gal3
- 3. CMP-SA, ST3GaI3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.
```

FIG. 38D

```
CHO, BHK, 293 cells, Vero or transgenic animal expressed α<sub>1</sub>-antitrypsin.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.
```

```
    CMP-SA-levulinate, ST3Gal3,
buffer, salt
    2. H<sub>4</sub>N<sub>2</sub>-PEG
```

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.
```

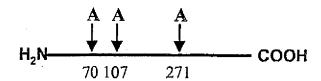
FIG. 38E

CHO, BHK, 293 cells, Vero expressed α_1 -antitrypsin. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.

1. CMP-SA, α2,8-ST

a-d, i, q-u (independently selected) = 0 or 1; e-h = 1; j-m (independently selected) = 0-20; v-y (independently selected) = 0.

FIG. 38F



$$\mathbf{A} \leftarrow \begin{bmatrix} (\operatorname{Fuc})_{i} & & & \\ (\operatorname{Fuc})_{i} & & & \\ (\operatorname{GlcNAc-(Gal)}_{a})_{e^{-}} (\operatorname{Sia})_{j} - (\operatorname{R})_{v} \\ (\operatorname{GlcNAc-(Gal)}_{b})_{f^{-}} (\operatorname{Sia})_{k} - (\operatorname{R})_{w} \end{bmatrix}_{s} \\ (\operatorname{GlcNAc-(Gal)}_{c})_{g^{-}} (\operatorname{Sia})_{l} - (\operatorname{R})_{x} \\ (\operatorname{R}')_{n} & & & & \\ (\operatorname{GlcNAc-(Gal)}_{d})_{h^{-}} (\operatorname{Sia})_{m^{-}} (\operatorname{R})_{y} \end{bmatrix}_{u} = \begin{bmatrix} (\operatorname{GlcNAc-(Gal)}_{a})_{e^{-}} (\operatorname{Sia})_{l} - (\operatorname{R})_{v} \\ (\operatorname{GlcNAc-(Gal)}_{d})_{h^{-}} (\operatorname{Sia})_{m^{-}} (\operatorname{R})_{y} \end{bmatrix}_{u} = \begin{bmatrix} (\operatorname{GlcNAc-(Gal)}_{a})_{e^{-}} (\operatorname{Sia})_{l} - (\operatorname{R})_{v} \\ (\operatorname{GlcNAc-(Gal)}_{d})_{h^{-}} (\operatorname{Sia})_{m^{-}} (\operatorname{R})_{y} \end{bmatrix}_{u} = \begin{bmatrix} (\operatorname{GlcNAc-(Gal)}_{a})_{e^{-}} (\operatorname{Sia})_{l} - (\operatorname{R})_{v} \\ (\operatorname{GlcNAc-(Gal)}_{d})_{h^{-}} (\operatorname{Sia})_{m^{-}} (\operatorname{R})_{v} \end{bmatrix}_{u} = \begin{bmatrix} (\operatorname{Sia})_{e^{-}} (\operatorname{Sia})_{l} - (\operatorname{R})_{v} \\ (\operatorname{GlcNAc-(Gal)}_{d})_{h^{-}} (\operatorname{Sia})_{m^{-}} (\operatorname{R})_{v} \end{bmatrix}_{u} = \begin{bmatrix} (\operatorname{Sia})_{e^{-}} (\operatorname{Sia})_{l} - (\operatorname{R})_{v} \\ (\operatorname{GlcNAc-(Gal)}_{d})_{l} - (\operatorname{Sia})_{l} - (\operatorname{R})_{v} \end{bmatrix}_{u} = \begin{bmatrix} (\operatorname{Sia})_{e^{-}} (\operatorname{Sia})_{l} - (\operatorname{R})_{v} \\ (\operatorname{GlcNAc-(Gal)}_{d})_{l} - (\operatorname{Sia})_{l} - (\operatorname{R})_{v} \end{bmatrix}_{u} = \begin{bmatrix} (\operatorname{Sia})_{e^{-}} (\operatorname{Sia})_{l} - (\operatorname{Sia})_{l} - (\operatorname{R})_{v} \\ (\operatorname{GlcNAc-(Gal)}_{d})_{l} - (\operatorname{Sia})_{l} - (\operatorname{Sia})_{l$$

a-d, i, n, p-u (independently selected) = 0 or 1.
e-h (independently selected) = 0 to 6.
j-m (independently selected) = 0 to 100.
v-y = 0;
R = modifying group, mannose, oligo-mannose;
R' = H, glycosyl residue, modifying group, glycoconjugate.

FIG. 38G

```
Insect or fungi cell expressed \alpha_1-antitrypsin.
a-d, f, h, j-m, s, u, v-y = 0;
e, g, i, q, r, t (independently selected) = 0 or 1.
```

- 1. GNT's 1,2,4,5, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal-PEG

```
a-i, q-u (independently selected) = 0 or 1; j-m = 0;
v-y (independently selected) = 1,
when e-h (independently selected) is 1;
R = PEG.
```

FIG. 38H

```
Yeast expressed \alpha_1-antitrypsin.

a-m=0; q-y (independently selected) = 0 to 1;

p=1; R (branched or linear) = Man, oligomannose.
```

- 1. Endoglycanase
- 2. Galactosyltransferase, UDP-Gal
- 3. CMP-SA-PEG, ST3Gal3

a-m, p-y = 0; n (independently selected) = 0 or 1; R' = -Gal-Sia-PEG.

FIG. 381

CHO, BHK, 293 cells, Vero expressed α_1 -antitrypsin. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.

- 1. CMP-SA-linker-Gal-UDP, ST3Gal3
- 2. Galactosyltransferase, transferrin treated with endoglycanase

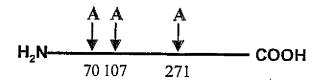
```
a-m, q-u (independently selected) = 0 or 1;

p = 1; n = 0;

v-y (independently selected) = 0 or 1;

R = linker-transferrin.
```

FIG. 38J



$$(Fuc)_{i}$$

$$A \leftarrow GlcNAc-Man$$

$$(R')_{p}$$

$$(R')_{q}$$

$$(GlcNAc-(Gal)_{a}]_{e} - (Sia)_{j} - (R)_{v}$$

$$[GlcNAc-(Gal)_{b}]_{f} - (Sia)_{k} - (R)_{w}$$

$$[GlcNAc-(Gal)_{c}]_{g} - (Sia)_{l} - (R)_{x}$$

$$[GlcNAc-(Gal)_{d}]_{h} - (Sia)_{m} - (R)_{y}$$

$$[GlcNAc-(Gal)_{d}]_{h} - (Sia)_{m} - (R)_{y}$$

a-d, i, n-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 4. j-m (independently selected) = 0 to 20. R = polymer; R', R" (independently selected) = sugar, glycoconjugate.

FIG. 38K

Yeast expressed alpha-1 antitrypsin.
a-h, i-m, p, q = 0;
R (independently selected) = mannose, oligomannose, polymannose;
r-u, v-y (independently selected) = 0 or 1; n, o = 1.

- 1. endoglycanase

a-h, i-o, q, r-u, v-y = 0; p = 1. R" = Gal-PEG.

FIG. 38L

Plant expressed alpha-1 antitrypsin. a-d, f, h, j-m, s, u, v-y = 0; e, g, i, q, r, t (independently selected) = 0 or 1; n=1; R' = xylose

- 1. hexosaminidase,
- 2. alpha mannosidase and xylosidase
- 3. GlcNAc transferase, UDP-GlcNAc-PEG

a-d, f, h, j-n, s, u, v-y = 0; e, g, i, r, t (independently selected) = 0; q = 1; R' = GlcNAc-PEG.

FIG. 38M

CHO, BHK, 293 cells, Vero, transgenic animal expressed α₁ antitrypsin.
a-h, i-o, r-u (independently selected) = 0 or 1;
p, q, v-y = 0.

1. CMP-SA-PEG, ST3Gal3

a-h, i-o, r-u (independently selected) = 0 or 1; p, q = 0; v-y (independently selected) = 0 or 1; R = PEG.

FIG. 38N